METHOD FOR QUANTIFYING LBP IN BODY FLUIDS

This application is a continuation-in-part of U.S. Serial No. 08/377,391 filed January 24, 1995, which is in turn a continuation-in-part of U.S. Patent Application Serial No. 08/186,811 filed January 24, 1994, all of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

The present invention relates to methods for determination of the presence of Lipopolysaccharide binding protein (LBP) in body fluid samples including blood samples.

Lipopolysaccharide (LPS) is a common component of the outer membrane of Gram-negative bacteria and is responsible for many of the pathologic effects associated with gram-negative bacterial infection and endotoxemia. Because of the association between bacterial infection and sepsis, attempts have been made to correlate serum/plasma levels of endotoxin with disease. Typically, endotoxin levels have been measured using the *Limulus* amebocyte lysate (LAL) assay, in which endotoxin initiates a coagulation cascade that can be measured physically, turbidimetrically, or spectrophotometrically, Despite these attempts, however, no reliable correlations between endotoxin levels and sepsis severity or outcome have been identified. This is most likely due to the fact that (i) endotoxin levels in septic patients are very low (> 10 pg/L), several serum proteins interfere with the proteolytic LAL cascade, (iii) endotoxin, once in contact with blood, can be "detoxified" by interaction with a variety of blood components, including high-density lipoprotein (HDL) and low-density lipoprotein (LDL) and (iv) endotoxin from different gram-negative organisms varies in its ability to trigger the LAL cascade. Thus, the absolute levels of endotoxin in a patient sample may not correspond to the actual concentrations of bioactive endotoxin present *in vivo*.

Two related proteins have been identified in humans and other animals that bind LPS with high affinity. These two proteins, Lipopolysaccharide binding protein (LBP), and bactericidal/permeability increasing protein (BPI) have roughly the same molecular weight and share 45% amino acid homology, yet exhibit distinct physiological differences. LBP is a 60 kD glycoprotein synthesized in the liver,

while BPI is found in the azurophilic granules of neutrophils. LBP is found in the serum of normal humans at levels of 5-10 μg/mL but can reach levels of 50-100 μg/mL in septic patients. Schumann et al., *Science*, *249*:1429 (1990) disclose the amino acid sequences and encoding cDNA of both human and rabbit LBP. Like BPI, LBP has a binding site for lipid A and binds to the LPS from rough (R-) and smooth (S-) form bacteria. Unlike BPI, LBP does not possess significant bactericidal activity. BPI has been observed to neutralize and inhibit the production of TNF resulting from interaction of LBP with LPS and CD14 on monocytes and macrophages. Marra et al., *J. Immunol. 148*: 532 (1992), Weiss et al., *J. Clin. Invest. 90*: 1122 (1992). In contrast, LBP is observed to enhance LPS-induced TNF production. Wright et al., *Science, 249*:1131 (1990). Thus, in contrast to BPI, LBP has been recognized as an immunostimulatory molecule. See, e.g., Seilhamer, PCT International Application WO 93/06228 which discloses a variant form of LBP which it terms LBP-β. Also of interest to the present invention are Ulevitch, PCT International Application WO 91/01639 which discloses, among other things, anti-LBP antibodies as an anti-sepsis therapeutic agent and U.S. Patent No. 5,245,013 which relates to LBP and discloses antibodies which immunoreact with a polypeptide having homology to LBP.

LBP has been characterized in the art as an "acute phase protein", that is one of many plasma proteins (such as C-reactive protein, fibrinogen and serum amyloid A) that increase in concentration in response to infectious and non-infectious tissue destructive processes. As such, it would be anticipated that LBP levels would be elevated in samples from patients suffering from a number of autoimmune diseases such as rheumatoid arthritis and lupus erythematosus.

Of interest to the present invention are disclosures related to the assaying of BPI activity in subjects. von der Mohien et al., Abstract, 13th International Symposium on Intensive Care and Emergency Medicine, Brussels (March 1993) discloses the results of assays for serum levels of BPI in patients with gram-negative sepsis and healthy subjects. The abstract disclosed that no BPI was detectable under the conditions of the assay in the serum of healthy subjects while circulating BPI was detected in all septic patients. Also of interest is the disclosure of co-owned and copending U.S. Patent Application Serial No. 08/175,276 filed December 29, 1993 which is a continuation-in-part of application 08/125,677 filed

September 22, 1993 the disclosures of which are hereby incorporated by reference. Those patent applications disclose that levels of BPI in blood plasma samples correlate with the presence or absence of sepsis while levels of BPI in blood serum samples do not. The patent applications teach that levels of BPI present in serum are not representative of endogenous extracellular levels of BPI in circulating blood while levels of BPI in plasma are.

Also of interest to the present invention are the disclosures of Leturcq et al., Keystone Tahoe Endotoxin Conference, March, 1-7, 1992 (Abstract) in which the generation of monoclonal antibodies to human LBP is reported. Also reported is the screening of normal human serum samples for the presence of LBP. LBP levels for normal serum samples were reported to range from 1 μg/mL to 24 μg/mL with an average of 7μg/mL. Further of interest is the disclosure of Richard Ulevitch at the American Society for Microbiology General Meeting in Atlanta, Georgia May 16-21 (1993) at which data was presented on LBP and soluble CD14 levels in the serum of septic and healthy individuals. The average soluble CD14 and LBP concentrations in the serum of healthy adults were 1 μg/mL and 7 μg/mL respectively. The average soluble CD14 and LBP concentrations in the serum of septic patients were reported to be 2 μg/mL and 55 μg/mL respectively.

Geller et al., *Arch. Surg., 128*: 22-28 (1993) disclose experiments in which the induction of LBP mRNA was studied in three models known to induce acute phase responses: (1) LPS injection; (2) *Corynebacterium parvum* injection; and (3) turpentine injection. The publication reports that LBP mRNA is induced during hepatic inflammation and suggest that LBP is an acute-phase protein important in regulating the *in vivo* response to endotoxin.

Gallay et al., *Infect. Immun., 61*:378-383 (1993) disclose that an acute phase response in mice injected with silver nitrate induced LBP synthesis, and that LBP levels increase approximately 10-fold over normal levels after an acute-phase response.

There exists a desire in the art for methods for determining the exposure of subjects to endotoxin and for distinguishing the effects of exposure to endotoxin from other acute phase physiologic responses. Also desired are methods

for diagnosing the presence or severity of gram-negative sepsis in a subject and for predicting the prognosis of a subject suffering from sepsis.

SUMMARY OF THE INVENTION

The present invention provides methods for specifically determining exposure of a subject to endotoxin by assaying for LBP. The invention further provides methods for screening for exposure to gram-negative bacterial endotoxin in an acute phase response in humans by assaying for LBP. Specifically, the method comprises the steps of determining the concentration of LBP in a sample of body fluid from the subject and correlating the concentration of LBP with a standard indicative of the exposure to endotoxin. Such standards can include a subjective standard for a given subject determined by LBP levels of that subject in a pretreatment state such as prior to undergoing surgery. Exposure to endotoxin as a consequence of such surgery can be determined by comparing post-surgical LBP levels with the standard established prior to surgery for that subject. Where access to a pretreatment standard level of LBP is not available for a given individual, objective standards based upon population or subpopulation averages may be applied for comparison. One such standard can be a concentration greater than approximately 15 μg/mL in human plasma or serum, as determined herein for LBP values in subjects suffering from numerous disease states. Subjects exhibiting LBP levels above that standard could presumptively be diagnosed as suffering from exposure to endotoxin while those having levels below that standard would not be. It is clear that alternative standards could be established depending upon the desired sensitivity and selectivity of an assay method and upon the subpopulation in which a given subject falls. For example, standards might be established at different levels for different ages, genders, ethnicities and underlying health conditions of various subpopulations. Moreover, it should be understood that standard levels will differ according to the identity of the particular body fluid which is assayed.

The invention further provides methods for diagnosing the presence or severity of sepsis in a subject comprising the steps of determining the concentration of LBP in a sample of body fluid from the subject and correlating the concentration of LBP with a standard indicative of the presence or severity of sepsis. The invention

further provides methods for predicting the prognosis of a subject suffering from sepsis comprising the steps of determining the concentration of LBP in a sample of body fluid from the subject and correlating the concentration of LBP with a standard indicative of the prognosis of a subject suffering from sepsis.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts the dose-response curves for rLBP, $rLBP_{25}$, rBPI and $rBPI_{23}$ in LBP sandwich assays;

Fig. 2 depicts LBP levels (mean ± standard error) in the plasma of healthy human subjects and human subjects suffering from various disease states;

Fig. 3 depicts LBP levels (mean ± standard error) in healthy subjects treated with LPS;

Fig. 4 depicts comparative survival in suspected gram-negative sepsis patients classified as having either high or low levels of plasma LBP; and

Figs. 5a-5c depict LBP, C-reactive protein (CRP) and fibrinogen levels (mean ± standard error) in healthy, rheumatoid arthritic and septic subjects.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods for quantifying the presence of LBP in body fluids including blood. While the assay can be used to determine the presence and quantity of LBP which has been administered therapeutically, it is particularly useful for quantifying the presence of endogenous LBP in circulating blood as an indication of exposure of a subject to endotoxin. Moreover, quantifying the presence of LBP is contemplated to be useful in diagnostic and prognostic methods for evaluating gram- negative sepsis patients.

The present invention provides a sandwich ELISA assay for human LBP which exhibits high assay sensitivity, high specificity, and excellent reproducibility. As used herein "LBP" quantitated according to assay methods includes native LBP, recombinant LBP, LBP fragments and analogs as well as other LBP proteins and protein products.

The amino acid and nucleotide sequence of recombinant LBP are set out in co-owned and copending U.S. Patent Application Serial No. 08/029,510 filed

June 17, 1993 as shown in SEQ ID NOS: 1 and 2 herein. A recombinant LBP amino-terminal fragment is characterized by the amino acid sequence of the first 197 amino acids of the amino-terminus of LBP as set out in SEQ ID NOS: 3 and 4 the production of which is described in co-owned and copending U.S. Patent Application Serial No. 08/079,510 filed June 12, 1993 the disclosure of which is incorporated herein. Such LBP protein products may be readily quantified using assays including immunological assays and bioassays in the subnanogram per mL range. Immunological assays capable of quantifying LBP are preferably carried out by enzyme linked immunosorbant (ELISA) sandwich assays but competitive assays and immunological assays utilizing other labelling formats may also be used. Preferred assays of the invention utilize anti-LBP antibodies, including monoclonal antibodies and affinity-purified rabbit polyclonal antibodies. Rabbit polyclonal anti-LBP antibodies may be prepared according to conventional methods using LBP as an immunogen. Non-immunological methods may also be used to assay for LBP. As one example, Ulevitch et al., U.S. Patent No. 5,245,013 disclose assay methods comprising binding of LBP to LPS and separating the complex by a centrifugation density gradient method. As another example, Geller et al., *Arch. Surg. 128*: 22-28 (1993) disclose LBP bioactivity assays in which IL-6 and TNF upregulation are measured.

Body fluids which can be assayed for the presence of LBP include whole blood with blood serum and blood plasma being preferred. Because LBP is a serum protein it is contemplated that it could be excreted and that analysis of LBP levels in urine may provide diagnostic and prognostic utility. The LBP immunoassays of the invention may also be used to determine the concentration of LBP in other body fluids including, but not limited to lung lavages, vitreous fluid, crevicular fluid, cerebrospinal fluid, saliva, sputum, ascites, amniotic fluid and synovial fluid.

Because LBP has been characterized as an "acute phase protein" it would be expected that LBP levels would be elevated in subjects suffering from autoimmune diseases. As one aspect of the present invention it has been found that LBP levels are not substantially elevated over normal in subjects with malignancies, immune diseases or syndromes, viral conditions and other conditions, such as, specifically, acute lymphoblastic leukemia (ALL), acute graft versus host disease

(aGvHD), chronic lymphocytic leukemia (CLL), cutaneous T-cell lymphoma (CTCL), type 1 diabetes, aplastic anemia (AA), Crohn's Disease, psoriasis, rheumatoid arthritis (RA), scleroderma, systemic lupus erythematosus (SLE), pregnancy and acquired immune deficiency syndrome (AIDS). In these patients with a condition not associated with endotoxin and thus not associated with elevated LBP levels, exposure to endotoxin is expected to produce a rise in LBP levels over the normal baseline LBP values for that condition. Thus, an elevation in LBP levels for these and other patient populations, like healthy subjects (*i.e.*, subjects with no evidence of disease), is expected to be diagnostic of an exposure to endotoxin.

The invention thus contemplates use of the LBP assay for distinguishing conditions associated with endotoxin from conditions (either acute phase or non-acute phase) not associated with endotoxin. The invention also contemplates the detection of exposure to endotoxin in healthy subjects or in patients with a condition not associated with endotoxin. The invention further contemplates multiple determinations of LBP levels of a subject over time, either to monitor for potential development of a condition associated with endotoxin or to monitor a subject for the severity or prognosis of a condition associated with endotoxin.

Certain subjects tentatively identified as suffering from gram-negative sepsis but ultimately identified as suffering from gram-positive sepsis also had elevated LBP levels. It is noted that translocation of bacteria and/or endotoxin from the gut into the bloodstream can occur in any infection. Thus, infections due to gram-positive bacteria or fungi may also lead to the presence of endotoxin or gram-negative bacteria in the blood and, therefore elevated levels of LBP.

The present invention is based in part upon the observation that serum and plasma levels of LBP directly correlate with a subject's exposure to biologically active LPS. Moreover, LBP levels appear to correlate with survival in suspected gram-negative sepsis patients. For example, subjects with levels of circulating LBP below 27.3 μg/mL (the median value for 58 subjects suffering from gram-negative sepsis) tended to have a greater 14 day survival than did those subjects with levels of LBP above that median. Further, for example, when a plasma LBP threshold level was set at 46 μg/mL, those subjects having a pretreatment LBP plasma level less than 46 μg/mL had a significantly greater survival rate ($p = 0.004$) over a 27 day period

than did those subjects having a pretreatment plasma LBP level greater than 46 μg/mL.

It is further contemplated by the invention that elevated levels of LBP may result from exposure to larger amounts of endotoxin, and may therefore be diagnostic of greater infection and/or endotoxemia severity. Elevated levels of LBP may also be used to indicate the suitability of using antibiotics directed against gram-negative bacteria or other therapeutic agents targeted directly to endotoxin such as BPI or anti-endotoxin antibodies including the monoclonal antibody E5.

Other aspects and advantages of the present invention will be understood upon consideration of the following illustrative examples. Example 1 relates to the preparation of affinity purified rabbit anti-BPI antibodies; Example 2 relates to the biotin labeling of such antibodies; and Example 3 relates to ELISA procedures utilizing such antibodies. Example 4 relates to the comparative immunoreactivity of rLBP, $rLBP_{25}$, rBPI AND $rBPI_{23}$. Example 5 relates to the measurement of rLBP spiked into pooled human plasma; and Example 6 relates to the comparison of LBP levels in human plasma and serum. Example 7 relates to the clinical correlations of endogenous LBP immunoreactivity with sepsis and other disease states in human plasma; and Example 8 relates to the effect of LPS administration on endogenous LBP levels in healthy subjects. Example 9 relates to clinical correlations between plasma LBP levels and survival in suspected gram-negative sepsis patients; and Example 10 relates to clinical correlations of acute phase proteins in healthy, rheumatoid arthritic and septic patients.

Example 1

PREPARATION OF AFFINITY PURIFIED RABBIT ANTI-rLBP ANTIBODY

According to this example affinity purified rabbit anti-rLBP antibody was prepared. Specifically, rLBP (20 mg) produced according to co-owned and copending U.S. Patent Application Serial No. 08/079,510 filed June 17, 1993, the disclosure of which is hereby incorporated by reference was coupled to 10 mL of cyanogen bromide-activated Sepharose 4B (Sigma Chemical Co., St Louis, MO) in 0.2 M bicarbonate, pH 8.6, containing 0.5 NaCl. Approximately

94% of the rLBP was coupled to the resin. Pooled antisera (125 mL) from two rabbits, immunized initially with $rLBP_{25}$ produced according to the methods of U.S. Patent Application Serial No. 08/079,510 filed June 17, 1993 and thereafter with rLBP, were diluted with an equal volume of phosphate buffered saline, pH 7.2 (PBS). A portion (50 mL) of the diluted antisera was passed through the 10 mL rLBP-Sepharose column; the column was then washed with PBS and bound antibodies were eluted with 0.1 M glycine, pH 2.5. Collected fractions were immediately neutralized with 1 M phosphate buffer, pH 8.0. Peak fractions were identified by measuring absorbance at 280 nm according to the method of Harlow et al., Antibodies: A Laboratory Manual, Cold Springs Harbor Laboratory Press, New York, p. 312 (1988). After several sequential column cycles, the affinity purified rabbit anti-LBP antibody was dialyzed against PBS-azide pH 7.2.

Example 2

PREPARATION OF BIOTIN LABELED RABBIT ANTI-rLBP ANTIBODY

In this example twenty milligrams of affinity purified rabbit anti-rLBP antibody produced according to the method of Example 1 was incubated with 2 mg of biotinamidocaproate N-hydroxysuccinimide ester (Sigma Chemical Co., St. Louis, MO) in 11 mL of 0.1 M sodium bicarbonate pH 8.3 for two hours at room temperature. Unconjugated biotin was removed and the alkaline buffer exchanged by fractionating the reaction mixture on a PD-10 column (Pharmacia Biotech Inc., Piscataway, NJ) equilibrated with PBS containing 0.1% sodium azide.

Example 3

ELISA PROCEDURE

Fifty microliters of affinity purified rabbit anti-rLBP antibody (2 μg/mL in PBS) were incubated overnight at 2-8°C (or alternatively, 1 hour at 37°C) in the wells of Immulon 2 (Dynatech Laboratories Inc., Chantilly, VA) microtiter plates. The antibody solution was removed and 200 μL of 1% non-fat milk in PBS (blocking agent) was added to all wells. After blocking the plates for

1 hour at room temperature, the wells were washed 3 times with 300 μL of wash buffer (PBS/0.05% Tween-20).

Standards, samples and controls were diluted in triplicate with PBS containing 1% bovine serum albumin, 0.05% Tween 20 (PBS-BSA/Tween) and 10 units/mL of sodium heparin (Sigma Chemical Co., St. Louis, MO) in separate 96-well plates. rLBP or $rLBP_{25}$ standard solutions were prepared as serial two-fold dilutions from 100 to 0.012 ng/mL. Each replicate and dilution of the standards, samples and controls (50 μL) was transferred to the blocked microtiter plates and incubated for 1 hour at 37°C. After the primary incubation, the wells were washed 3 times with wash buffer. Biotin-labeled rabbit anti-LBP antibody was diluted 1/2000 in PBS-BSA/Tween and 50 μL was added to all wells. The plates were then incubated for 1 hour at 37°C. Subsequently, all wells were washed 3 times with wash buffer. Alkaline phosphatase-labeled streptavidin (Zymed Laboratories Inc., San Francisco, CA) was diluted 1/2000 in PBS-BSA/Tween and 50 μL was added to all wells. After incubation for 15 minutes at 37°C, all wells were washed 3 times with wash buffer and 3 times with deionized water and the chromogenic substrate p-nitrophenylphosphate (1 mg/mL in 10% diethanolamine buffer) was added in a volume of 50 μL to all wells. Color development was allowed to proceed for 1 hour at room temperature, after which 50 μL of 1 N NaOH was added to stop the reaction. The absorbance at 405 nm was determined for all wells using a Vmax Plate Reader (Molecular Devices Corp., Menlo Park, CA).

The mean absorbance at 405 nm (A_{405}) for all samples and standards (in triplicate) were corrected for background by subtracting the mean A_{405} of wells receiving only sample dilution buffer (no LBP) in the primary incubation step. A standard curve was then plotted as A_{405} versus ng/mL of rLBP or $rLBP_{25}$. The linear range was selected, a linear regression analysis was performed and concentrations were determined for samples and controls by interpolation from the standard curve.

Example 4

COMPARATIVE IMMUNOREACTIVITY OF rLBP, $rLBP_{25}$, rBPI AND $rBPI_{23}$

In this example, the immunoreactivity of rLBP, $rLBP_{25}$, rBPI and $rBPI_{23}$ were compared in the BPI sandwich ELISA to determine possible immunologic cross-reactivity. Despite considerable sequence homology between LBP and BPI (see, e.g., Schumann et al., *Science, 249*:1429 (1990), the results illustrated in Fig. 1 show that, on a mass basis, $rBPI_{23}$ produced a signal which was approximately 3 orders of magnitude lower than that of $rLBP_{25}$ and rLBP, while rBPI produced a signal that was approximately 5 orders of magnitude lower than that of rLBP and $rLBP_{25}$. For example, a concentration of 100,000 ng/mL (100 μg/mL) of rBPI or 400 ng/mL $rBPI_{23}$ generated a signal which was equal to that produced by 0.8 ng/mL of rLBP or 0.4 ng/mL of $rLBP_{25}$. These results demonstrate minimal cross-reactivity of the antibody with BPI and confirm the specificity of the assay for LBP.

Example 5

MEASUREMENT OF rLBP SPIKED INTO POOLED HUMAN PLASMA

In this example, the recovery of rLBP in human blood fluids was evaluated by examining pooled human plasma spiked with different concentrations of rLBP and then frozen and thawed prior to measurement in the sandwich ELISA. Recovery of spiked LBP was defined as the amount of LBP measured in spiked human plasma samples minus the concentration in the unspiked control, divided by the actual amount spiked in the sample. The fraction recovered was multiplied by 100 and the results were expressed as a percentage of the input concentration. Recovery of different concentrations of rLBP spiked into pooled human plasma samples averaged 68% and ranged from 59% at 42 μg/mL to 78% at 168 μg/mL. Table I summarizes the recovery data for each LBP spiked plasma sample.

TABLE I

Recovery of rLBP Spiked into Pooled Citrated Human Plasma

Amount Spiked (μg/mL)	Amount Measured (μg/mL)	Amount Recovered (μg/mL)	Percent Recovery
0	2.47	---	---
10.5	9.85	7.38	70%
21	16.1	13.63	65%
42	27.3	24.83	59%
84	60.8	58.33	69%
168	133	130.53	78%
		Mean Recovery	68%

Example 6

COMPARISON OF PLASMA AND SERUM LBP LEVELS

According to this example concentrations of LBP in the serum and plasma of healthy subjects were assayed and compared utilizing the sandwich ELISA assay according to Example 3. Plasma concentrations of LBP were found to be essentially the same as serum concentrations for LBP when the plasma volume was corrected for dilution (dividing by a factor of 0.85) resulting from the addition of anticoagulant. Plasma concentrations in normal human subjects were found to be 3.1 μg/mL (S.D. 0.9 μg/mL) or 3.7 μg/mL (S.D. 1.1 μl/mL) corrected, compared with 3.7 μg/mL (S.D. 0.9 μg/mL) for serum.

Example 7

CLINICAL CORRELATIONS OF
ENDOGENOUS LBP IMMUNOREACTIVITY IN HUMAN PLASMA

In this example endogenous LBP immunoreactivity was measured in human plasma or serum samples collected from a variety of subjects suffering from gram-negative sepsis and a variety of other clinical conditions. Specifically, plasma samples of healthy individuals (59 subjects), and individuals diagnosed

with gram-negative sepsis (390 subjects) were assayed for LBP levels. Serum samples of individuals with acute lymphoblastic leukemia (ALL) (6 subjects); acute graft versus host disease (aGvHD) (8 subjects); chronic lymphocytic leukemia (CLL) (9 subjects); cutaneous T-cell lymphoma (CTCL) (12 subjects); type 1 diabetes (13 subjects); aplastic anemia (AA) (16 subjects); Crohn's Disease (8 subjects); psoriasis (13 subjects); rheumatoid arthritis (RA) (86 subjects); scleroderma (4 subjects), and systemic lupus erythematosus (SLE) (10 subjects) were assayed for LBP levels. The results are shown in Fig. 2.

In another experiment, LBP immunoreactivity was also measured in plasma samples from healthy non-pregnant women (18 subjects) and in plasma samples from age-matched pregnant women in the third trimester of gestation with no evidence of active infection (18 subjects). The mean result for the healthy non-pregnant women was 3.8 μg/mL (ranging from 1.7 to 7.7 μg/mL), which was comparable to that of the healthy individuals assayed earlier, and the mean result for the pregnant women was 10.5 μg/mL (ranging from 4.6 to 22.7 μg/mL).

In a further experiment, levels of LBP as well as C-reactive protein (an acute phase protein) were measured in plasma samples from patients diagnosed with AIDS, with no concomitant infection. The mean LBP value was 5.33 $\pm$ 2.31 μg/mL (ranging from 2.1 to 10.3 μg/mL) and the mean CRP level was 5.34 $\pm$ 6.99 μg/mL (ranging from 0.25 to 18.3 μg/mL).

While LBP levels among subjects diagnosed as suffering from gram-negative sepsis were elevated it was found that LBP levels were not substantially elevated over normal in subjects with acute lymphoblastic leukemia, acute graft versus host disease, chronic lymphocytic leukemia, cutaneous T-cell lymphoma, type 1 diabetes, aplastic anemia, Crohn's Disease, psoriasis, rheumatoid arthritis, scleroderma, systemic lupus erythematosus (SLE), pregnancy and AIDS. Accordingly, the LBP assay of the invention is valuable for distinguishing conditions associated with endotoxin from other acute phase conditions or non-acute phase conditions not associated with endotoxin.

Example 8

THE EFFECT OF LPS ADMINISTRATION ON ENDOGENOUS LBP LEVELS IN HEALTHY SUBJECTS

In this example, the effect of LPS administration on endogenous LBP immunoreactivity in healthy human subjects was determined. Specifically, healthy subjects were monitored utilizing the LBP sandwich assay for changes in LBP plasma levels at various time points after intravenous administration of 4 ng/kg LPS (16 subjects) or in control subjects (2) not receiving LPS. The results illustrated in Fig. 3 show the change in mean plasma LBP concentration with time. For those subjects treated with LPS LBP levels began to rise about 6 hours after LPS administration. Peak LBP plasma levels were observed in most subjects between 10 to 12 hours after the LPS administration. The average increase from baseline to peak LBP level was approximately 3-fold. Over this time period the mean LBP levels in control subjects remained within normal range (approximately 5 μg/mL).

It is contemplated that additional analysis will illustrate the correlation of LBP levels in body fluids with the symptoms of exposure to endotoxin and that LBP levels will be diagnostic and prognostic of disease states resulting from exposure to endotoxin.

It is contemplated that additional analysis will illustrate the correlation of LBP levels with symptoms of bacterial infections, endotoxemia and sepsis including conditions associated with sepsis including DIC and ARDS.

Example 9

CLINICAL CORRELATIONS BETWEEN PLASMA LBP LEVELS AND SURVIVAL IN SUSPECTED GRAM-NEGATIVE SEPSIS PATIENTS

Correlations between plasma LBP levels and survival in suspected gram-negative sepsis patients were compared using data obtained from the septic subjects described in Example 7. In this case, a standard LBP concentration was set at 46 μg/mL and patients with suspected gram-negative sepsis were classified as having either high (>46 μg/mL) or low (<46 μg/mL) LBP plasma levels as measured in pretreatment samples. As shown in the data presented in Fig. 4,

those subjects having low pretreatment plasma levels of LBP had a significantly greater survival rate ($p = 0.004$) over a 27 day period than did those subjects having a high pretreatment plasma LBP level. These data show the utility of assaying LBP levels and comparing them to a standard LBP value for predicting the prognosis of subjects suffering from sepsis.

Example 10

CLINICAL CORRELATIONS OF ACUTE PHASE PROTEINS IN HEALTHY, RHEUMATOID ARTHRITIC, AND SEPTIC PATIENTS

Plasma levels of LBP, C-reactive protein (CRP) and fibrinogen were measured in small groups of healthy, rheumatoid arthritic and septic patients with the results shown in Figs. 5a (LBP levels), 5b (CRP levels) and 5c (fibrinogen levels). The results show that relative to healthy subjects, mean fibrinogen levels were elevated approximately 2.5 fold for both rheumatoid arthritic and septic subjects. Relative to healthy subjects, mean CRP levels were found to be elevated approximately 40-fold for rheumatoid arthritic subjects and 200-fold for septic subjects. In contrast, and consistent with the results in Example 7, mean LBP levels were only slightly increased (less than 2-fold) for rheumatoid arthritis subjects while the mean LBP levels were increased by more than 6 fold for septic subjects.

Numerous modifications and variations in the practice of the invention are expected to occur to those skilled in the art upon consideration of the foregoing description of the presently preferred embodiments thereof. Consequently, the only limitations which should be placed upon the scope of the present invention are those which appear in the appended claims.